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Airway Obstruction Is Increased in *Pneumocystis*-Colonized Human Immunodeficiency Virus-Infected Outpatients[▽]

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We investigated the relationship of *Pneumocystis* colonization, matrix metalloprotease levels in sputum, and airway obstruction in a cohort of human immunodeficiency virus (HIV)-infected outpatients. *Pneumocystis*-colonized subjects had worse obstruction of airways and higher levels of matrix metalloprotease-12 in sputa, suggesting that *Pneumocystis* colonization may be important in HIV-associated chronic obstructive pulmonary disease.

With the advent of highly active antiretroviral therapy (HAART) for human immunodeficiency virus (HIV), opportunistic pulmonary infections have decreased, but other lung diseases may be increasing as patients live longer exposed to both HIV and pulmonary insults, such as smoking. For example, HIV-infected subjects have an accelerated form of emphysema (3, 4) and a high prevalence of chronic obstructive pulmonary disease (COPD) (2). The causes of this accelerated COPD are not known, but it has been hypothesized that subclinical pulmonary infections could be involved (3, 4).

Pneumocystis jirovecii is a microbial pathogen that may be important in the pathogenesis of HIV-associated COPD. *Pneumocystis* colonization as detected by nested PCR is common in HIV-infected persons, particularly those who smoke (12). *Pneumocystis* colonization has also been found in non-HIV-infected subjects with COPD and is an independent predictor of the severity of airflow obstruction (13). In nonhuman primates infected with simian immunodeficiency virus/HIV, *Pneumocystis*-colonized animals develop significant airflow obstruction, while *P. jirovecii*-negative, simian immunodeficiency virus/HIV-infected animals do not (14). In addition, normal mice exposed to both cigarette smoke and *Pneumocystis* demonstrate physiologic and histological changes associated with COPD and emphysema (1).

Pneumocystis colonization might contribute to the development of COPD through the release of endogenous proteases and stimulation of protease release from the host lung. The predominant matrix metalloproteinases (MMPs) postulated to be involved in COPD are MMP-1 (interstitial collagenase), MMP-2 (gelatinase A), MMP-9 (gelatinase B), and MMP-12 (macrophage metalloelastase) (18). The levels of host-derived MMP-2 and MMP-9 increase during acute *Pneumocystis* pneumonia (PCP) and may contribute to the parenchymal destruction or airway remodeling seen (16, 19), but the role of MMPs in *P. jirovecii* colonization has not been explored.

We performed a prospective cohort study to test the hypothesis that *Pneumocystis* colonization is associated with airway obstruction in HIV-infected outpatients and to determine the relationship of colonization to MMP levels in sputum.

Subjects. Subjects were HIV-infected outpatients at the University of Southern California (USC) attending a routine health care visit. Those subjects experiencing acute symptoms, such as fevers, cough, or increasing shortness of breath, within the past month were excluded. Subjects with a known history of asthma were also excluded. The USC IRB approved the study. All subjects provided informed consent.

Data collection. Clinical data were collected prospectively by interview and medical record review. Demographic data included age, gender, and race/ethnicity. Medical data included history of previous pneumonia, including PCP; medication use, including antiretrovirals; PCP prophylaxis; and bronchodilator use. Smoking and intravenous drug use histories were collected, and subjects were questioned about current respiratory symptoms (cough, shortness of breath, or dyspnea on exertion). Laboratory data included $CD4^+$ T-lymphocyte count and plasma HIV viral levels documented within the previous 6 months.

Spirometry. Spirometry was performed according to American Thoracic Society (ATS) criteria after the administration of 200 μg albuterol (5). Percent predicted values for forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) were calculated using standard reference equations (7).

Sputum induction and oral washes. Sputum induction was performed by inhalation of 3% hypertonic saline for 20 min. Oral washes were collected by having subjects gargle with 0.9% sterile saline for 1 min. Sputa and oral washes were mixed with an equal volume of 0.1% dithiotreitol and processed as described previously (9).

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Determination of *Pneumocystis* colonization status. DNA extraction was performed on sputa and oral washes using a DNeasy kit (Qiagen, Valencia, CA). *Pneumocystis* colonization was determined by nested PCR of the mitochondrial large subunit rRNA as previously described (9). DNA extraction and PCR were carried out in separate rooms, and all PCRs were performed in a UV box. Positive and negative controls were included in each reaction mixture. A subject was considered *P. jirovecii*-colonized if PCR of either induced sputum or oral wash demonstrated human *Pneumocystis* by DNA sequencing in duplicate reactions. Real-time PCR was performed at the mitochondrial large subunit rRNA locus for 7 of the 11 *Pneumocystis*-colonized subjects.

Measurement of MMPs in sputum. Sputum supernatants were analyzed for levels of MMP-1, MMP-2, MMP-7, MMP-8, MMP-9, and MMP-12 by using a human MMP multianalyte profiling Fluorokine kit (R&D Systems, Minneapolis, MN) and a Luminex-based Bio-Plex multiplex suspension protein array system (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. The concentrations of each MMP were determined by using Bio-Plex Manager version 4.1.1 software.

Statistical analysis. Stata 8 (Stata Corporation, College Park, TX) was used for analysis, and significance determined for a P value of ≤ 0.05. Continuous variables were described using mean and standard deviation or median and range depending on normality of data. Univariate analyses were performed to determine clinical variables related to *P. jirovecii* colonization using either t test/Wilcoxon rank sum or chi-square/Fisher's exact test. The relationship of *P. jirovecii* colonization to airway obstruction was determined by comparing the postbronchodilator FEV_1 percent predicted value and FEV_1/FVC ratio for *P. jirovecii*-colonized and *P. jirovecii*-negative subjects. Fisher's exact test was used to determine the association of *P. jirovecii* colonization and having a diagnosis of COPD, defined as a postbronchodilator FEV_1/FVC ratio below 0.70 (10). An adjusted odds ratio was computed by controlling for smoking history as defined by current versus former or never smoker. Linear regression was used to adjust similarly for the effects of smoking on the FEV_1 percent predicted value and FEV_1/FVC ratio. Linear regression adjusting for smoking status was used to compare the MMP levels in sputa of *P. jirovecii*-colonized and *P. jirovecii*-negative subjects. Analyses of airway obstruction were repeated using the *P. jirovecii* copy number as the predictor variable.

Forty-two HIV-infected subjects were enrolled. Most (85.7%) were male, and the average age was 46.5 years (range, 31.8 to 69.2). The majority of subjects (78.6%) were receiving HAART. Eleven subjects (26.2%) were *P. jirovecii* colonized. The average *P. jirovecii* copy number in colonized subjects was 5.3 copies/ml of DNA (range, 1.9 to 507). *P. jirovecii*-colonized subjects were similar to *P. jirovecii*-negative subjects in gender, age, and ethnicity (Table 1). There were no significant differences between *P. jirovecii*-colonized and *P. jirovecii*-negative subjects in percentage of current smokers, average pack year history, or history of PCP, bacterial pneumonia, or tuberculosis. *P. jirovecii*-colonized subjects were significantly less likely to have ever taken trimethoprim-sulfamethoxazole. There was a trend for more *P. jirovecii*-colonized subjects to report any respiratory symptom or to use bronchodilators.

TABLE 1. Characteristics of *Pneumocystis*-colonized and *Pneumocystis*-negative subjects

Characteristic[a]	Result[b] for:	
	P. jirovecii-positive subject(s) (n = 11)	*P. jirovecii*-negative subjects (n = 31)
Male	8 (72.7)	28 (90.3)
Age [mean yrs (SD)]	46.3 (8.1)	46.6 (8.8)
Ethnicity		
White	1 (9.1)	3 (9.7)
African-American	6 (54.6)	13 (41.9)
Hispanic	4 (36.4)	15 (48.4)
CD4 count [median no. of cells/μl (range)]	501 (155–756)	411 (4–1059)
HIV viral load [median no. of copies/ml (range)]	399 (<50–178,000)	399 (<50–267,727)
HAART use	7 (63.6)	26 (83.9)
Prior PCP	2 (18.2)	4 (12.9)
Prior bacterial pneumonia	1 (9.1)	4 (12.9)
Prior tuberculosis	2 (18.2)	3 (9.7)
PCP prophylaxis	1 (9.1)	8 (25.8)
TMP-SMX use, ever[c]	2 (18.2)	22 (71.0)
Current smoker	5 (45.5)	12 (38.7)
Pack year history [median (range)]	6.0 (0.3–12)	4.5 (0.1–26)
Respiratory symptoms	4 (36.4)	5 (16.1)
Bronchodilator use	2 (18.2)	0 (0)

[a] SD, standard deviation; TMP-SMX, trimethoprim-sulfamethoxazole.
[b] Values are given as no. (%) unless indicated otherwise in the first column.
[c] Odds ratio of 0.10 for *P. jirovecii*-colonized compared to *P. jirovecii*-negative subjects; 95% confidence interval, 0.02 to 0.58; P = 0.008.

P. jirovecii-colonized subjects differed significantly from *P. jirovecii*-negative subjects in their spirometric values. The FEV_1 percent predicted values and FEV_1/FVC ratios were lower in *P. jirovecii*-colonized subjects (Fig. 1A). This relationship persisted after adjustment for smoking status (P value of 0.02 for adjusted FEV_1 value, and P value of 0.009 for adjusted FEV_1/FVC ratio). *P. jirovecii*-colonized subjects were also significantly more likely to have a clinical diagnosis of obstruction, based on an FEV_1/FVC ratio of <0.70 with an adjusted odds ratio of 8.8 (95% confidence interval, 1.3 to 59.8, and P value, 0.03) (Fig. 1B). *P. jirovecii* copy number was not significantly associated with spirometric values (P value of 0.35 for FEV_1 percent predicted value, and P value of 0.50 for FEV_1/FVC ratio).

Univariate analysis demonstrated that the levels of MMP-12 in sputa were significantly higher in *P. jirovecii*-colonized than in *P. jirovecii*-negative subjects (205.7 pg/ml versus 47.7 pg/ml; P = 0.01) (Fig. 1C). This relationship persisted after adjustment for smoking history (adjusted P value, 0.02). There were no differences in *P. jirovecii*-colonized compared to *P. jirovecii*-negative subjects in levels of MMP-1, MMP-2, MMP-7, or MMP-8. Median levels of MMP-9 were higher in *P. jirovecii*-negative subjects than in *P. jirovecii*-colonized subjects (125,430.3 pg/ml versus 63,603.8 pg/ml; P = 0.02), but this relationship did not persist after adjustment for smoking status (adjusted P value, 0.06).

We found that *P. jirovecii* colonization predicted a lower absolute FEV_1 value and FEV_1/FVC ratio, as well as clinical airway obstruction, independent of smoking history. MMP-12, a protease thought to be important in COPD pathogenesis (6, 11), was increased in those with *P. jirovecii* colonization. Al-

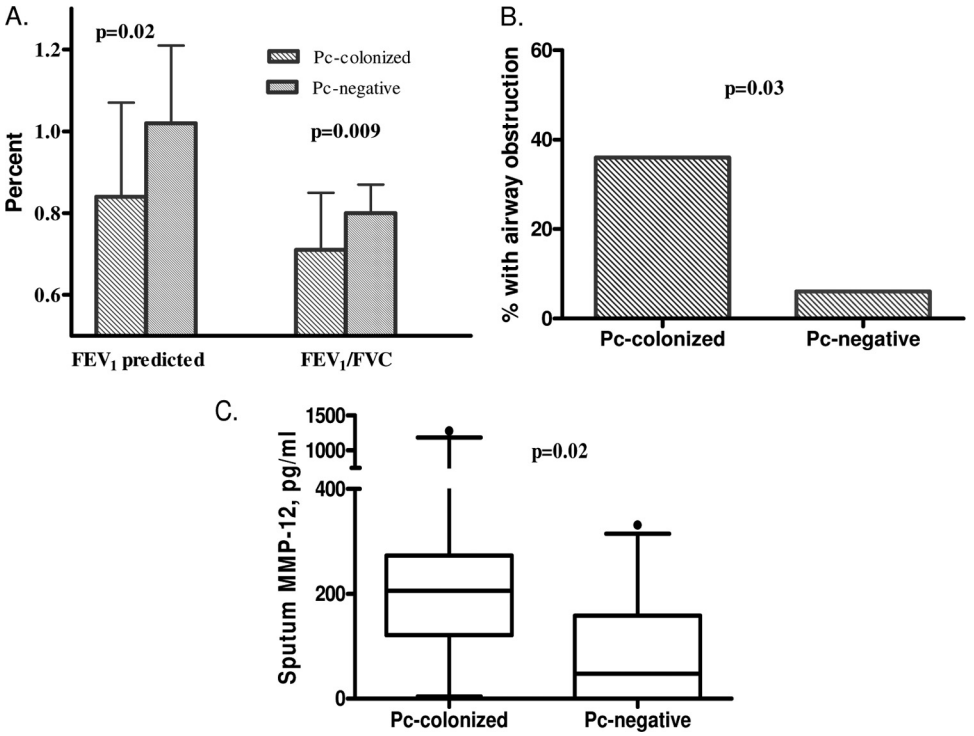

FIG. 1. Relationship of *Pneumocystis* colonization to spirometry values and MMP levels in sputum. (A) Mean FEV_1 percent predicted and FEV_1/FVC ratio according to *P. jirovecii* colonization status (error bars represent standard deviation, adjusted for smoking status). (B) Percent of subjects with clinical airway obstruction, based on an FEV_1/FVC ratio of <0.70, according to *P. jirovecii* colonization status (*P* value adjusted for smoking status). (C) Box-and-whiskers plot of median MMP-12 levels in sputum according to *P. jirovecii* colonization status (whiskers represent 10th and 90th percentiles; horizontal lines represent median values; boxes represent interquartile ranges; *P* value adjusted for smoking status). Pc, *P. jirovecii*.

though causation cannot be determined from the current study, these findings lend additional support to the hypothesis that *Pneumocystis* is involved in the pathogenesis or progression of airway obstruction in HIV infection and suggest that MMP-12 may be important in the disease process.

HIV-infected subjects are at increased risk for development of COPD, particularly if they smoke (1–3). It has been postulated that latent or subclinical infections might play a role in the development of HIV-associated COPD, but no studies to date have explored this hypothesis. Previous work examining *P. jirovecii* colonization and COPD in the non-HIV-infected population demonstrated that *P. jirovecii* colonization is increased in COPD and correlates with disease severity (13, 15). Animal models also support a role of *P. jirovecii* colonization in COPD pathogenesis, as COPD-like changes have been reported in both a murine and a nonhuman primate model of *P. jirovecii* colonization (1, 14).

The presence of *Pneumocystis* in the lungs, even at low levels, produces inflammatory changes similar to those seen in COPD, with increases in neutrophils, macrophages, and $CD8^+$ lymphocytes. Inflammatory cells are important in releasing MMPs, and it is possible that *P. jirovecii* colonization thus results in MMP release. MMP-12 is particularly important in COPD, as it is necessary for the development of emphysema in cigarette smoke-exposed mice, and COPD patients have increased MMP-12 levels in bronchoalveolar lavage samples (6, 11). Furthermore, MMP-12 may also contribute to the COPD airway remodeling, as it is associated with myofibroblast migration (17). We found that *P. jirovecii* colonization was associated with increased MMP-12 levels in sputum, suggesting that MMP-12 release might be one mediator linking *P. jirovecii* colonization and COPD. Although other MMPs have been linked to COPD, we did not find that they were elevated with *P. jirovecii* colonization, potentially because HIV infection itself can alter MMP levels (8).

There are several limitations of the study. Our sample size was small, and we may have lacked power to detect important correlates of *P. jirovecii* colonization or the ability to detect a relationship of airflow obstruction with *P. jirovecii* copy number. We did not test diffusing capacity for carbon monoxide or perform computed tomography scanning, limiting our ability to assess different COPD phenotypes. Because our subjects were recruited from a single center and were relatively healthy, these results may not be generalizable to other HIV-infected populations.

In summary, *P. jirovecii* colonization is associated with worse airway obstruction in HIV-infected subjects and correlates with increased levels of MMP-12 in sputum. This work builds on previous studies by prospectively examining healthy HIV subjects and directly measuring airway obstruction, thereby adding to the evidence that *P. jirovecii* colonization may be linked to COPD development. If *P. jirovecii* colonization is found to play a causal role in some cases of HIV-associated

COPD, antibiotic therapy might ameliorate disease progression.

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The authors have no conflict of interest.

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